*TB MED 292

DEPARTMENT OF THE ARMY TECHNICAL BULLETIN

DETERMINATION OF CHOLINESTERASE ACTIVITY: MANUAL AND AUTOMATED METHODS

Headquarters, Department of the Army, Washington, D.C.

30 May 1975

1. Introduction. a. Cholinesterase enzymes in human blood cells and in plasma are inhibited in varying degrees by organophosphorus nerve agents and many common insecticides. A method for measuring the resulting reduction in the level of blood cholinesterase activity is one means of establishing whether or not an individual has been exposed to such compounds.

b. Provisions of this publication are subject of an international agreement (STANAG 2870). When amendment, revision or cancellation of this publication is proposed which affect or violate the international agreement concerned, the preparing activity will take appropriate reconciliation action through international Standardization channels.

2. Purpose and Scope. *a.* As numerous methods have been developed for determining blood cholinesterase activity values, many different ways of reporting units of cholinesterase activity resulted. The purpose of this bulletin is to recommend manual and automated methods for measuring blood cholinesterase activities to assure accuracy, precision and uniformity in order that one laboratory will be able to compare their enzyme activity values with those obtained by another laboratory.

b. This bulletin (1) describes in detail two manual methods for red blood cell cholinesterase, one manual method for plasma cholinesterase activity, and one automated method for measuring red cell and plasma cholinesterase activity. The original manual method for red cell cholinesterase (ref 1) requires 1 hour. A modification of the original method requires 17 minutes (ref 2). The automated method requires more expensive equipment and more extensive personnel training (ref 3). In this procedure cholinesterase activities of red cell and plasma can be measured at a rate of 30 samples per hour.

(2) Describes controls for the daily monitoring of manual and automated procedures.

(3) Provides equations for converting cholinesterase activity units of the manual procedures to those of the automated procedure, and vice versa.

(4) Describes procedures for collecting and handling blood samples.

(5) Provides a list of laboratory equipment and reagents.

*This bulletin supersedes TB MED 292. 30 August 1974.

3. Standard **Manual Electrometric (60 Minute)** Method for Measuring Red **Cell Cholinesterase** Activity. This method of testing requires the equipment listed below. Use of any other type of equipment used in this required standardized method of testing will invalidate and cause test to give false readings.

a. Apparatus and Equipment (app B).

(1) **pH** meter.

(a) Coleman Model **37A,** accurate to 0.01 **pH** units, line or battery operated.

(*b*) Beckman, Model **G pH** meter, accurate to 0.02 **pH** units, battery operated.

(*c*) A **pH** meter accurate to 0.01 **pH** units.

(2) Electrodes for **pH** meter (combination electrode):

(a) A. **H.** Thomas, Catalog **#4858-L15,** for either Coleman Model 37A or Beckman Model G.

(*b*) **Markson** T-T, Model 808 for Coleman **37A pH** meter.

(3) Centrifuge for separating red blood cells from plasma.

(4) Analytical balance, accurate to 0.1 milligram.

(5) Constant temperature bath which can be regulated at **25°C** ± 0.5".

(6) A timer or stop watch.

(7) 5.0 ml beakers.

(8) Glass stirring rods (about 2 inches long, about 3 mm diameter).

(9) Twenty-microliter, disposable, capillary pipettes (many commercial sources, one is Scientific Products).

(10) Suction tubing and holders for capillary pipettes.

b. Reagents.

(1) Buffer for red blood cells: in 90 ml distilled water dissolve 412.4 mg sodium **barbital,** 54.5 mg potassium dihydrogen phosphate and 4.47 gm potassium chloride. Add **0.1N** hydrochloric acid, approximately 2.1 ml, until a **pH** value of 8.1 at **25°C** is obtained. Make to 100 ml total volume. Store in a refrigerator at 4°C. Discard when the **pH** is less than 7.90.

(2) Substrate for red blood cell determinations: **0.11M** acetylcholine chloride, 2.0 gm in **100** ml of distilled water. Add 7.5 ml of distilled water to a preweighed vial containing 150 mg acetylcholine chloride (Sigma Chemical Company). Shake the vial gently to make certain the small amount of acetylcholine chloride adhering to the inside of the cap dissolves. Make up a **fresh** solution after one week's storage.

c. Procedure for Collection and Handling of Blood Samples.

(1) ***Collection of blood.*** Specimens for cholinesterase assay are obtained from a blood sample collected either in a lavender-top **EDTA** Vacutainer (Becton-Dickinson) or in a **green**-top heparin Vacutainer. preferably the former. Blood may also be withdrawn by **syringe** into a test tube containing either the anticoagulant EDTA (2 mg per ml of blood) or USP heparin (20 units per ml of blood), preferably the former.

(2) Preparation of sample. Separate the red cells from plasma by centrifugation for PO minutes at 2500 rpm. Remove the plasma from the red cells. Mix the packed red cells prior to sampling.

(3) ***Storage.*** Whole blood can be kept at room temperature for 8 hours with little loss of cholinesterase activity. For longer storage periods, separate plasma and red cells, and keep refrigerated at 4°C.

(4) Shipping. Ship separated plasma and red blood cells in wet ice, not dry ice. If neither anticoagulant nor centrifuge is available, allow the blood to clot. Withdraw as much of the serum as possible and transfer to a clean tube. Stopper tightly and ship in wet ice. Do not ship coagulated whole blood.

d. Standardization of pH Meter. Use a reference phosphate buffer standard of about **pH** 7.0. If the electrode is new or has been used with alkaline solutions, immerse it in **0.1N** hydrochloric acid for **1/2** hour prior to use.

e. Assay Procedure for Measuring Red Cell Cholinesterase, Using the 60-Minute Manual Method. Red blood cells, which have been separated from plasma by centrifugation, are drawn to the **0.02-ml** mark (20μl) of a capillary pipette and excess blood is wiped from the outside of the pipette. The red cells are gently expelled into a 5-ml beaker containing a glass stirring rod and 1.0 ml of distilled water. Be certain to keep the pipette tip below the surface of the water. Rinse pipette at least 5 times with the water in the beaker. Mix the blood and water gently with the stirring rod and allow the mixture to stand approximately 5 minutes for completion of **hemolysis.** Leave the stirring rod in the beaker for future mixing. To the beaker add 1.0 ml of red blood cell buffer and stir. Place the beaker in a water bath at 25°C. After 5 minutes read and record the initial **pH** (pH_1) to the nearest 0.01 **pH** unit. Then add 0.2 ml of the acetylcholine chloride solution with mixing and record the time, **using** a timer or stop watch. Allow the reaction to proceed for 1 hour. Stir the final mixture 2 or 3 times during this l-hour period. hfter the l-hour period, record the final **pH** (pH_2). Subtract the pH_2 reading from the pH_1 reading (pH_1–pH_2)and record this value as ΔpH units per hour (ΔpH/hr).

f. Comments and Suggestions. Blank values due to hydrolysis of acetylcholine chloride at

25°C are negligible. **Buffer solution can be** stored frozen in plastic containers. **The buffer** is stable for 6 months. Be certain to bring the buffer solution to room temperature **(25°C ± 3°C)** before using. Use eel cholinesterase **(para 7a)** as a red **blood** cell control to establish precision. An acceptable coefficient of variation (precision) of red blood cell samples that are measured by the **ΔpH** method is ± 4 percent **(para** 8).

g. Normal Red Blood Cell Cholinesterase Ranges. The normal red blood cell cholinesterase values found by this method given in **ΔpH/** hr units are: mean value 0.72; 95 percent range of normal values fall between 0.58 **and 0.86. See** paragraph **9a(1)** for recording results.

4. Modified Manual **Electrometric (1 'I-Minute) Method for Measuring Red Cell Cholinesterase Activity.** ***a. Apparatus and Equipment*** (see app B). Identical to the standard manual **60-minute** method, with one exception: a **100-μl** capillary pipette is used in place of the **20-μl** pipette.

b. Reagents. Identical to the standard manual **60-minute** method **(para 3b);** 0.02 percent **saponin**: dissolve 20 mg saponin in 100 ml of water. Prepare fresh daily.

c. Procedure8 for Collection and Handling of Blood Samples. Identical to the standard manual **60-minute** method **(para 3c).**

d. Standardization of pH Meter. Identical to the standard manual 60-minute method **(para 3d).**

e. Assay Procedure for Measuring Red Cell Cholinesterase Activity, Using the 17-Minute Manual Method. Practice the pipetting of red cells before performing this assay. Pipette 1 ml of 0.02 percent saponin solution into a **5-ml** beaker. Red blood cells are drawn to the **100-μl** mark of a capillary pipette and the excess blood wiped off. Place the tip of the pipette below the surface of the saponin solution and gently expel the red cells into the beaker. Rinse the capillary pipette about 10 times with the **saponin** solution in the beaker. Stir the solution with a glass stirring rod to obtain complete hemolysis and allow to equilibrate for 5 minutes. Leave the stirring rod in the beaker **for** future mixing. To the beaker add 1.0 ml of red blood cell buffer and stir. Place the beaker in a water bath at **25°C.** After **5** minutes record the initial **pH** (pH_1) to the nearest 0.01 **pH** unit. **Then** add 0.2 ml of the **acetylcholine** chloride solution with mixing and **record** the time using a timer or stop watch. Allow the reaction to proceed for 17 minutes. Stir 1 or 2 times during this **17-minute** period. After the **17-minute** period record the final **pH** (pH_2). Subtract the pH_2 reading from the pH_1 reading ($pH_1 - pH_2$) and record this result as **ΔpH** units **(ΔpH/hr).**

f. Comment8 and Suggestions. **The resulting ΔpH** value for the **17-minute** method is identical to the **ΔpH** value obtained by the **Mb-minute** methdd. The modified **ΔpH** method **has** a high degree of correlation with the **standard ΔpH** method (r = 0.94). Blank values **due** to hydrolysis of acetylcholine chloride at **25°C are** negligible. Buffer solution can be stored frozen **in** plastic containers. The buffer is stable for 6 months. Be certain to bring the buffer solution to room temperature **(25°C ± 3°C) before using.** Use eel cholinesterase **(para 7a) as a red blood** cell control to establish precision. An **acceptable** coefficient of variation (precision) of red blood cell samples that are measured by the **ΔpH** method is ± 4 percent **(para** 8).

g. Normal Red Blood Cell Choline&erase Ranges. The normal red blood cell cholinesterase values found by this method **given** in **ΔpH/** hr units are: mean value 0.72; 95 percent **range** of normal values fall between 0.58 and 0.86. See paragraph **9a(1)** for recording results.

5. Standard Manual Electrometric (60-Minute) Method for Measuring Plasma Cholinesterase Activity. ***a. Apparatus and Equipment*** **(app B).** Same as for manual method for measuring red cell cholinesterase activity **(para 3a).**

b. Reagents.

(1) Buffer for plasma: in 90 ml distilled water dissolve 124 mg sodium barbital, 13.6 mg potassium dihydrogen phosphate, and 1.75 gm sodium chloride. Add **0.1N** hydrochloric **acid,** approximately 0.9 ml until a **pH** of 8.0 at **25°C** is obtained. Make to 100 ml total volume. Store in a refrigerator at **4°C.** Discard when the **pH** is less than 7.90.

(2) Substrate for **plasma: 0.165M acetylcholine** chloride, 3.0 gm in 100 ml of distilled water. Add 5.0 ml of distilled water to a preweighed vial containing **150** mg acetylcholine **chloride** (Sigma Chemical Company). Shake the **vial** gently to make certain the small amount of acetylcholine chloride adhering to the inside of the cap dissolves. Make up a fresh solution after one week's storage.

c. Procedure for Collection and Handling of Blood Samples. Same as for manual method for measuring red cell cholinesterase activity **(para 3c).**

d. Standardization of pH Meter. **Same as for** manual method for measuring red cell **cholinesterase** activity **(para 3d).**

e. Assay Procedure for Plasma Cholinesterase Using ***the 60-Minute Manual Method. Plasma,*** which has been separated by **centrifugation,** is drawn to the **0.02-ml** mark **(20μl) of a capillary** pipette and excess plasma is **wiped from the**

outside of the pipette. The plasma is gently expelled into a 5-ml beaker containing a stirring rod and 1.0 ml of distilled water. Be certain to keep the tip of the pipette below the surface of the water. Rinse pipette at least 3 times with the water in the beaker. Mix the plasma and water gently with the stirring rod, Leave the stirring rod in the beaker for future mixing. To the beaker add 1 ml of plasma buffer and stir. Place the beaker in a water bath at 25° C. After 5 minutes read and record the initial pH (pH_1) to the nearest 0.01 pH unit. Then add 0.2 ml of the acetylcholine chloride solution with mixing, and record the time, using a timer or stop watch. Allow the reaction to proceed for 1 hour. Stir the final mixture 2 or 3 times during this l-hour period. After the l-hour period, record the final pH (pH_2). Subtract the pH_2 reading from the pH_1 reading ($pH_1 - pH_2$) and record this value as ΔpH/hr units.

f. Comments and Suggestions. Blank values due to hydrolysis of acetylcholine chloride at 25°C are negligible. Buffer solution can be stored frozen in plastic containers. The buffer is stable for at least 6 months. Be certain to bring the buffer solution to room temperature (25°C ± 3°C) before using. Use Monitrol or Hyland lyophilized serum (para 7*b*) as a plasma control to establish precision. An acceptable coefficient of variation (precision) of plasma samples that are measured by the ΔpH method is ± 4 percent (para 8).

g. Normal Plasma Cholinesterase Range. The normal plasma cholinesterase values found by the method given in ΔpH/hr units are: mean value 0.95; 95 percent range of normal values fall between 0.61 and 1.28. See paragraph 9*a*(2) for recording results.

6. Automated Method for **Measuring Red Cell and Plasma Cholinesterase.**

a. Apparatus and Equipment (app B). The analytical system consists of the following modules from Technicon Instruments Corporation: Sampler II, proportioning pump II, 37°C heating bath, 37°C dialyzer with type C membrane, colorimeter with 15-mm flow cell and 420-nm filters, recorder with optical density paper. A manifold is constructed and the system assembled as shown in flow diagram (fig 1). Other equipment needed is: centrifuge, analytical balance, timer or stop watch, and disposable plastic sample cups.

b. Reagents.

(I) Diluent: saponin, 0.01 percent. Dissolve 0.1 gm of saponin in 1 liter of distilled water containing 5 ml of Brij 35. Prepare fresh daily.

(2) Buffer: Tris (hydroxymethyl) aminomethane (Tris) buffer, pH 8.2, 0.05M. Dissolve 6.640 gm of sodium chloride and 6.050 gm of Tris in approximately 950 ml of distilled water containing 5 ml of Brij 35. Adjust pH to 8.2, using 6N hydrocloric acid. Dilute to 1 liter with water. This is stable for 2 months in a refrigerator at 4°C.

(3) Color reagent: 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) in buffer, 8.4 x 10-4M. Dissolve 0.3326 gm DTNB in 1 liter of Tris buffer, pH 8.2. This is stable for 2 months at 4°C.

(4) Substrate: acetylthiocholine iodide (AcSChI), 1.005 x 10-2M. Dissolve 0.2905 gm of AcSChI in 100 ml of distilled water. The final concentration in the sample stream of the automated system is 2 x 10-3M. This is stable for 2 weeks at 4°C.

(5) Standard: reduced glutathione (GSH), 100 μmoles/ml. 0.3073 gm of GSH in 10 ml of distilled water. Prepare fresh daily.

(6) Brij 35. Wetting agent obtained from Technicon Instruments Corporation, Tarrytown, New York 10591.

c. Procedure for Collection and Handling of Blood Samples. Identical to the procedure used in the manual methods (para 3*c*).

d. Automated Procedure for Plasma *and Red Blood Cell Cholinesterase.*

(1) Manifold: the plasma or red blood cell sample is mixed first with the diluent (saponin solution), resulting in a 1:26.8 dilution. An aliquot of the diluted sample is mixed with DTNB buffer. The substrate is combined with the sample stream before the mixture enters the heating bath. After incubation the sample stream is dialyzed against buffer. The absorbance of the 'recipient stream is measured and recorded. The chemical reactions which occur in the sample stream are shown below:

$$\text{Acetylthiocholine} + \text{Cholinesterase} \xrightarrow[\text{(Sample)}]{} \text{Thiocholine} + \text{Acetic Acid}$$

$$\text{Thiocholine} + \text{DTNG} \longrightarrow \text{Colored Product}$$

(2) Assay: while all reagents are being pumped through the system, adjust the colorimeter to give a baseline reading of 0.01 absorbance (optical density or O.D.) units. Each cup containing standard or sample should be followed by a cup containing saline to provide acceptable separation of peaks. Therefore, every other space on the sampler plate should contain a cup of saline. The sampler operates at a rate of 60 samples/hour, resulting in an effective rate of 30 samples/hour. Assay a series of glutathione standards containing 6.25, 12.5, 25, 50 and 100 μmoles/ml, respectively, prior to blood samples (fig 2).

(3) Blank: transfer the substrate line to a

ccntainer of distilled water and readjust the recorder baseline to 0.01 O.D. prior to performing blank assays. Rerun samples as described above. It is unnecessary to perform individual blank assays on all samples since there is little variation in blank absorbency values: plasma, 0.003 ± 0.001 and red blood cell, 0.071 ± 0.005. During an 8-hour working day the average absorbence value obtained from 6 plasma or red blood cell blank assays may be used to correct the assay absorbence value of each individual sample.

e. Automated Procedure for Samples Taken From Finger or Earlobe (Capillary Samples). If an assay must be performed when sample volume is small, a manual dilution (1:26) of either red blood cells or plasma may be prepared as follows:

0.5 ml of 0.01% saponin diluent + 20 µl of sample

Sample this dilution directly into the analytic system by connecting the sample line A to the aliquot pump tube B (fig 1). Discontinue pumping reagents in that portion of the manifold inclosed by dashed line. Glutathione standards must also be diluted 1:26. These dilutions may be assayed at the rate of 60/hr without saline cups between samples. The 20 µl sample for analysis can be obtained from a finger or earlobe puncture by the technique described by Stubbs and Fales (ref 4).

f. Calculation of Results for Automated Procedure. Prepare a standard curve (fig 3) each ay by plotting glutathione concentrations vs O.D. (peak reading minus baseline reading) readings in linear graph paper. Convert the sample ΔO.D. (Assay O.D. minus blank O.D.) reading to corresponding micromole per milliliter value by means of the standard curve. This value must be divided by the heating bath time, the length of time the substrate is in contact with the sample, measured each day by stop watch from the point at which substrate joins the sample stream until the sample stream exits from the dialyzer plate (approximately 6 min). Cholinesterase activity is expressed as micromoles of substrate hydrolyzed by 1 ml of sample/minute of incubation at 37°C. The calculation to obtain micromoles per milliliter per minute is shown below:

Example: FIRST RED CELL SAMPLE (see fig 2)

	Peak	*Baseline*		
Assay O.D.	0.460	0.010	=	0.450
Blank O.D.	0.078	0.010	=	0 068
ΔO.D.				0.382

0.382 = 73.2 µmoles/ml from standard curve (fig 3). The micromole per milliliter value must be divided by incubation time. When incubation time is 6.00 minutes, enzyme activity is $\frac{73.2}{6.00}$ or 12.2 µmoles/ml/min. Use eel cholinesterase (para 7*a*) as a red blood cell control and Monitrol or Hyland lyophilized serum (para 7*b*) as a plasma control to establish precision (see para 8). An acceptable precision for both red cell and plasma cholinesterase is ± 3 percent.

g. Normal Red Blood Cell and Plasma Cholinesterase Ranges and Comments. The normal cholinesterase values obtiined by the automated method given in international units (micromoles/ml/min) are:

		95 percent range of normal values
Red blood cell	12.6	1X2-15.1
Plasma	4.2	2.7-5.7

The red blood cell and plasma coefficients of correlation (r) for the standard (60 min) ΔpH and automated method are 0.94 and 0.96, respectively. See paragraph 9*a* for recording results.

Note. A reaction occurs between acetylthiocholine and the standard glutathione to form acetylglutathione and thiocholine (ref 5). This reaction results in a mixture of two sulfhydryl compounds, glutathione and thiocholine. Both react equivalently with DTNB in the sample stream prior to dialysis. Consequently, the standard calibration curve as shown in figure 3 with glutathione is proper.

7. Controls for Manual and Automated Cholinesterase Methods.

a. *Red Blood Cell Control Standards*

(1) Recommended source of enzyme: eel acetylcholinesterase Type VI, lyophilized powder, 1 mg solid, 100-200 micromolar units. Sigma Chemical Company, St. Louis, MO 63178.

(2) Procedure: Make up a solution of acetylcholinesterase, type VI, in Tris buffer, pH 8.2, as described in paragraph 6*b*(2), such that the final enzyme solution consists of 10-15 micromolar units/ml of buffer, i.e., if the activity of the purchased acetylcholinesterase is reported by the manufacturer to be 200 micromolar units, add about 15 ml of buffer solution to each mg of enzyme that is weighed out. This stock solution is stored in a refrigerator or freezer. Prepare a fresh red cell enzyme control solution after 2-week storage in either a refrigerator or freezer. Red blood cell enzyme activity units for the standard will be established by a selected referee laboratory.

b. Plasma Control Standard-Lyophilized Serum.

(1) Recommended sources of enzyme: Q-PAK-Chemistry Control Serum, Analyses, Lot No. 3699 R 002A1, Hyland, Costa Mesa, California; Monitrol-Human Control Serum, Lot No. LTD 103 A, B, Dade, Miami, Florida.

(2) Procedure: Reconstitute lyophilized serum as directed by the supplier. Prepare a

fresh plasma control solution after **2-week** storage in' **a** refrigerator or freezer.

(3) Comments. Use the reconstituted serum daily as a control for **plasma cholinesterase** assays. Serum enzyme activity units will be established by a selected referee laboratory.

8. Quality Control (Precision). a. Use eel cholinesterase **(7*a*)** for establishing the precision (expressed as coefficient of variation) of red blood cell cholinesterase and serum **cholinesterase (7*b*(1))** for establishing the precision of the plasma cholinesterase at the beginning of an &hour working day.

b. If the precision value for five replicate control samples is outside the acceptable limit, repeat the procedure. If an acceptable value is not obtained on repetition, then an inquiry into the cause of the excessive deviation should be made.

c. In addition, run one control sample for every ten unknown samples. Evaluate the precision of these internal controls. If the coefficient of variation exceeds the recommended precision limit, then the results should be suspect. The source of error should be determined and the unknown samples rerun.

9. Interconversion of Manual and Automated Cholinesterase Activity Units.

a. The enzyme activity unit of the **ΔpH** methods can be changed to the enzyme activity unit of the automated method by use of the formulae below:

(1) Red blood cell micromoles **(μM) ml/min** units = 0.66 + 16.6 times **ΔpH/hr** units.

Example of Red Blood Cell Conversions

Problem 1. The ΔpH/hr value for a red blood cell determination was found to be 0.75. What is the corresponding automated value in μM/ml/min?

Answer: **automated value = 0.66 + 16.6 x 0.75**
= 0.66 + 12.45
= 13.11 μM/ml/min

Problem 2. The automated red cell value was found to be 12.60μM/ml/min. What is the ΔpH/hr value?

Answer: **12.60 = 0.66 + 16.6 × ΔpH value**

$$\frac{12.60 - 0.66}{16.6} = \Delta pH/hr$$

0.72 = ΔpH/hr

(2) Plasma **μM/ml/min** units = 0.09 + 4.35 times **ΔpH/hr** units

Example of Plasma Conversion

Problem 1. The ΔpH/hr value for plasma was found to be 1.19. What is the corresponding value in μM/ml/min?

Answer: automated value = 0.09 + 4.35 × 1.19
= 0.09 + 5.18
= 5.27

Problem 2. The automated plasma value was found to be 3.67. What is the ΔpH/hr value?

Answer: **3.67 = 0.09 + 4.35 x ΔpH/hr**

$$\frac{3.67 - 0.09}{4.35} = \Delta pH/hr$$

0.82 = ΔpH/hr

10. Recommendations and Comments. ***a.*** The **17-minute** manual method should be used when 1-15 red cell cholinesterase samples are determined in an B-hour working day.

b. The 60-minute manual method should be used when 16-75 red cell and/or plasma **cholinesterase** samples are determined in an **8-hour** working day.

c. The automated method should be used when more than 75 determinations of red cell and/or plasma cholinesterase samples are determined in an **8-hour** working day.

d. The standard **(60-minute)** manual and automated methods can be performed with **20**-microliter samples of either red blood cells or plasma. A method for performing a 60-minute **ΔpH** red cell or plasma choline&erase determination from blood drawn from a finger or earlobe is given in reference 4.

e. User Comments. Users of this bulletin are encouraged to submit recommended changes or comments to improve the methods (equipment, accuracy, short cuts,-etc.). Reasons should be given for each comment to insure understanding and complete evaluation. Comments on a DA Form 2028 (Recommended Changes to Publications and Blank Forms) should be sent **to The Surgeon General, Department of the *Army,* Washington, D.C. 20310.**

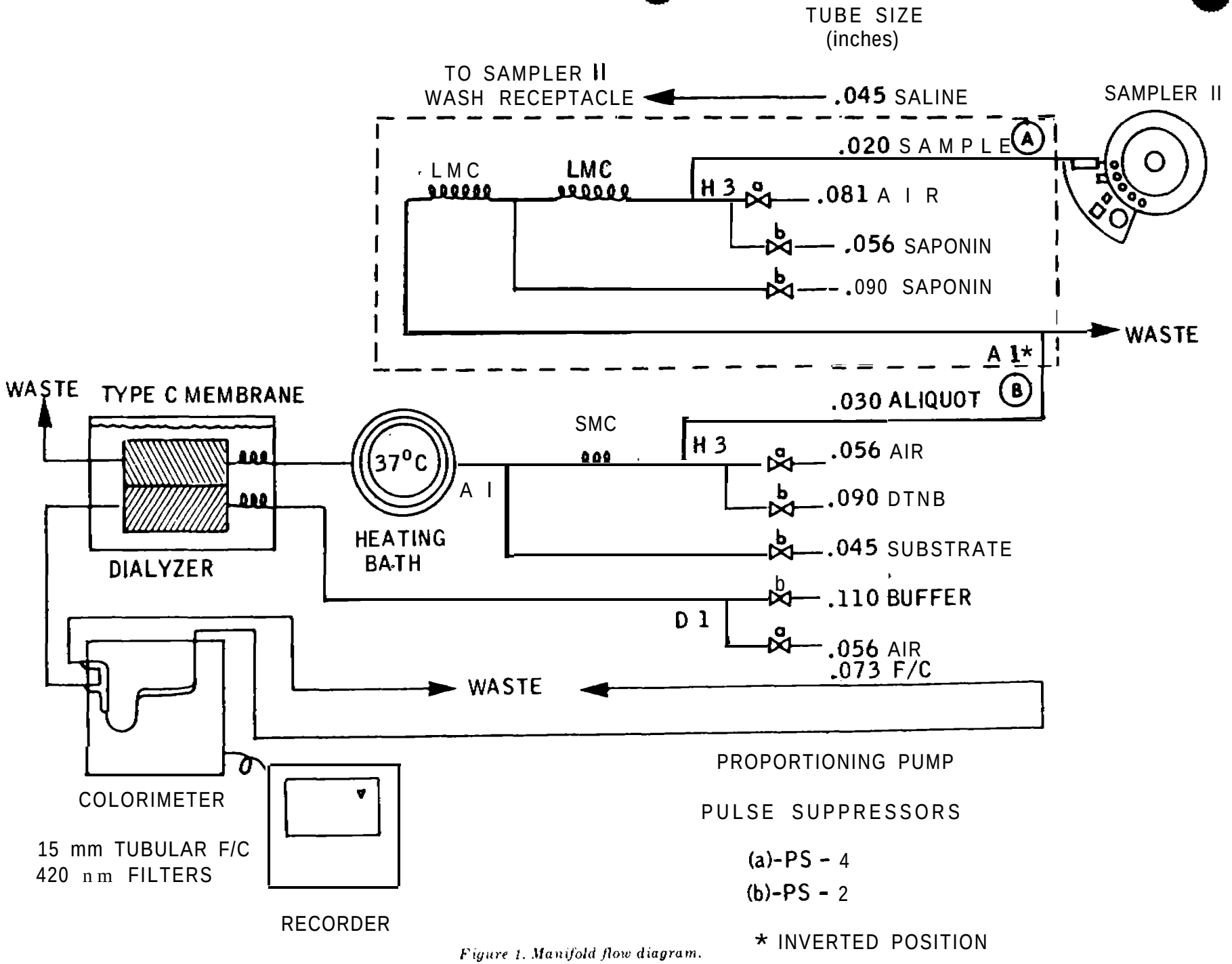

Figure 1. Manifold flow diagram.

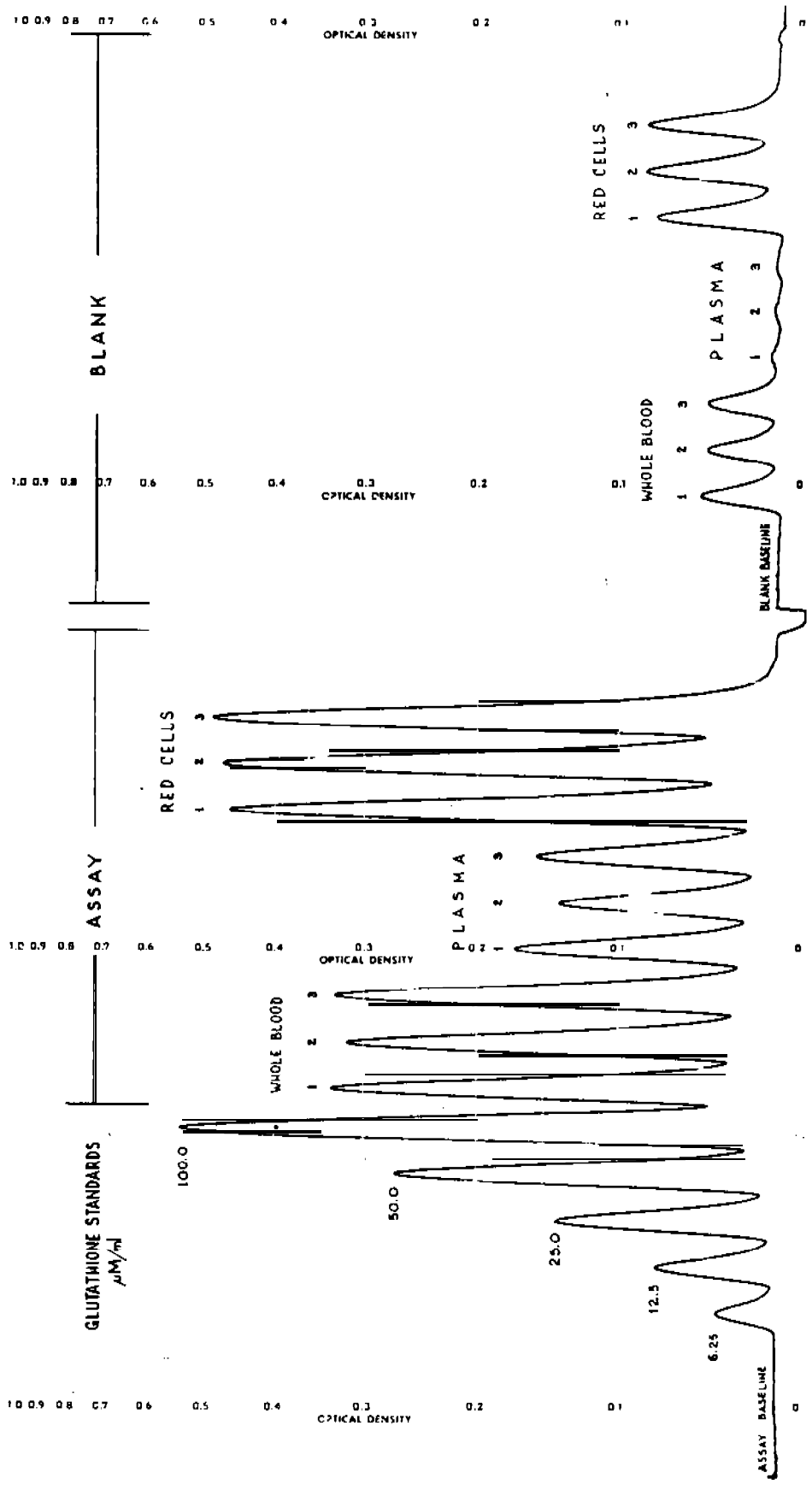

Figure 2. Strip Chart recording.

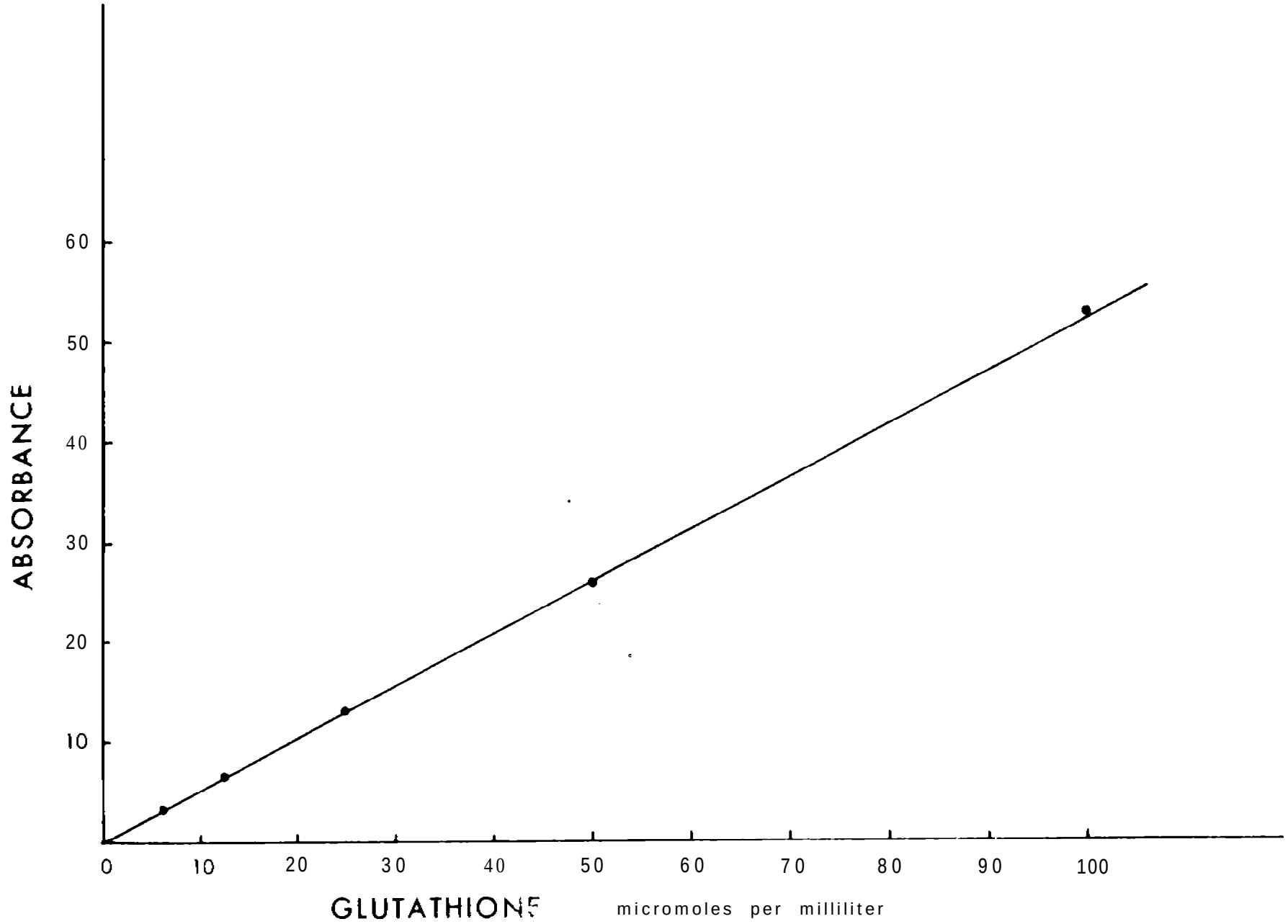

Figure 3. Glutathione standard calibration curve.

APPENDIX A

REFERENCES

1. Michael, H.O. An Electrometric Method for Determination of Red Cell and Plasma Cholin-e&erase Activity. J. Lab. Clin. Med. *34*, 1564 (1949).

2. Ellin, R.I., Burkhardt, B.H. and Hart, R.D. A time-Modified Method for Measuring Red Blood Cells Cholinesterase Activity. Arch. Environ. Health 27, 48(1973).

3. Groff, W.A., Kaminskis, A., and Ellin, R.I. Edgewood Arsenal Technical Report 4628. A Versatile Automated System for Measuring Blood Cholinesterase Activity. October 1971.

4. Stubbs, J.L. and Fales, J.T. A Capillary Sampling Technique for Determination of Cholinesterase Activity in Red Cells and Plasma. Amer. J. Med. Techn. *26*, 25(1960).

5. Ellin, R.I., Groff, W.A., and Kaminskis, A. An Error-Producing Interaction in an Automated Method for Measuring Cholinesterase Activity in Blood. Clin. Chem. 18, 1009(1972).

Notes 1. Civilian publications and books are available in Army Medical Libraries as set forth in paragraphs 99 and 101, AR 40-2.

2. The references listed above are the many articles that culminate the research and findings that gave reason to make this TB Med the accepted standardized method of determination for the Base Line Cholinesterase for humans. If the user of this TB Med desires further information or copies of the above listed references, inquiry should be made to the US Army Biomedical Laboratory, Edgewood Arsenal, Maryland 21010.

APPENDIX B

APPARATUS AND EQUIPMENT

Manual Methods (For Standard and Modified ΔpH Methods)

1. pH meter
   a. Beckman Model G, battery operated, accurate to 0.02 pH units, FSC* #6830-431-4750.
   b. Coleman Model 37A, line and battery operated, accurate to 0.001 pH units, FSC #6630-105-8648.
2. Bateries for pH meter
   a. BA 34, FSC #6135-120-1017.
   b. BA 35, FSC #6135-120-1016.
   c. BA 230/U, FSC #6135-100-0459.
3. Electrodes for pH meter
   a. Recommended: combination electrode, A.H. Thomas, Catalog #4094 L 15; Markson T-T electrode, Catalog #808, Markson Science Supply.
   b. Buffer solution, pH 7, to standardize pH meter, FSC #6630-072-924 1.
   c. Potassium chloride solution, saturated for calomel electrode, Fisher Scientific Co., Catalog #So-p-138.
4. Vacutainer, green or lavender top. tubes, Becton-Dickinson, Rutherford, New Jersey 07070.
5. Centrifuge for separating red blood cells from plasma: centrifuge, clinical, horizontal type head, to provide speeds to 3,000 rpm, FSC #6640-930-9034.
6. Analytical balance
   a. With chainweight device, sensitivity to 0.1 mg, FSC #6670-401-3005.
   b. Mettler Model H10T, sensitivity to 0.1 mg, Fisher.' Scientific, Catalog #1-908-70; Scientific Products, B-125.5; A.H. Thomas, 1839-J-10.
7. Constant temperature water bath which can be regulated to 25°C ± 0.5". (Heater control useful only if ambient temperature is less than 25°C. If ambient temperature is greater than 25°C, *use* ice or cold water to lower temperature.)
8. Timers
   Electric timer, Scientific Products C6516.
8. Beakers. glass, 5 ml, with spout, FSC #6640-291-6877.
10. Glass stirring rods (about 2 inches long, about 3 mm diameter), FSC #6640-290-0154. These are 7 inches long, cut to Z-inch lengths.
11. Pipettes, capillary, disposable, 20 and 100 μl, Scientific Products, Catalog #P4518-20.
12. Barbital sodium, Fisher Scientific, Catalog #B-22.
13. Potassium dihydrogen phosphate, FSC #6810-137-5000.
14. Sodium schloride. FSC #6810-264-6592.
15. Acetylcholine chloride, preweighted, Sigma Chemical Company, Catalog #420-750.
16. Saponin, Fisher Scientific Co., Catalog #S-672.
17. Tris (hydroxymethyl) aminomethane (Tris), Fisher Scientific Co., catalog #T-370.
18. Potassium chloride, FSC #6810-264-6545.

Automated Method

1. Colorimeter with 15 mm tubular flow cell and voltage stabilizer (105-A501), Part #112-X000-02, price list XC-15.
2. Dialyzer, temperature controlled (37°C), part #105-A000-01, price list AC-15, dialyzer plates, Lucite.
3. Heating bath (37°C), double coil (1.6 mm I.D. x 40 ft.), part #105-A101-01.
4. Proportioning pump II, two-speed, part #133-A009.
5. Recorder, single pen, part #011-A000-01.
6. Sampler II module, part #127-A000.
equipment listed below is available from Technicon Instruments Corp., Contract #GS-00S-05468, FSC Group 66 (1 May 73-30 Apr 74).
7. Standard pump tube, I.D. .020 in., part #116-0532-05 (also available from Kradko, part #TM--51).
8. Standard pump tube, I.D. .030 in. Technicon part #116-0532-07. Gradko part #TM-7.
9. Standard pump tube, I.D. .045 in. Technicon part #116-0532-10. Gradko part #TM-10.
10. Standard pump tube, I.D. .056 in. Technicon part #116-0532-12. Gradko part #TM-12.
11. Standard pump tube, I.D. .073 in. Technicon part #116-0532-14. Gradko part #TM-15.
12. Standard pump tube, I.D. .081 in. Technicon part #116-0532-15. Cradko part #TM-16.
13. Standard pump tube, I.D. .090 in. Technicon part #116-0532-16. Gradko part #TIM-17.
14. Standard pump tube, I.D. .110 in. Technicon part #116-0532-18. Gradko part #TM-19.

* FSC is Federal Stock Catalog, FSC 6810 Identification Listing (IL) and Management Data Listing (MDL).

15. Transmission tubing, 1/16 in. x 1/8 in. Technicon part #116–0528–01. Gradko part #TA–3.

16. Sleeving, heavy wall, 1/8 in. ;Ts 1/4 in. Technicon part #562–0005. Gradko part #TA-2.

17. Nipple, connector N5. Technicon part #116-0002-01. Gradko part #NC-l.

18. Nipple, connector N6. Technicon part #116-000441. Gradko part #NC-2.

19. Nipple, connector N7. Technicon part #116–0005-01. Gradko part #NC–3.

20. Nipple, connector N8. Technicon part #116–0003–01. Gradko part #NC-4.

21. Connector, T, T1. Technicon part #116–020041. Gradko part #1–6.

22. Connector, H, D1. Technicon part #116–0203-01. Gradko part #3-2.

23. Connector, special H3. Technicon part #116–0211. Gradko part #5–1.

24. Pulse suppressor,. PS4. Technicon part #116–B044–01.

25.. Pulse suppressor, PS2. Technicon part #116–B044–02.

26. Coil, mixing, 14 turns, 2.4 mm I.D. Technicon part #105–0086. Gradko part #23–1.

27. Coil, mixing, 28 turns, 2.4 mm I.D. Technicon part #105–0087. Gradko part #23–10.

28. Polystyrene sample cups, 0.5 ml. Technicon part #127–0093. Gradko part #GG101C.

29. Chart paper, log, R0487. Technicon part #011-0015. Gradko part #GG0487.

30. Dialyzing membranes, type C. Technicon part #105–1058.

Reagents

FSC #6810–NSN

1. Saponin, Fisher Scientific Co., Catalog #S–672. Sigma Co., Catalog #S1252.

2. Tris (hydroxymethyl) aminomethane, Fisher Scientific Co., Catalog #T-370. Sigma Co., Catalog #T1503.

3. 5,5′-dithiobis-(2-nitrobenzoic acid), Sigma Co., Catalog #D8130. Calbiochemical Co., Catalog #322123.

4. Acetylthiocholine iodide, Sigma Chemical Co., Catalog #A5751. Fisher Scientific Co., Catalog # 10587.

5. Glutathione, reduced form, Sigma Co., Catalog #G4251. Calibiochemical Co., Catalog #3541.

6. Brij 35. 30 percent solution, Technicon Instruments Corp., Catalog #T21–0110–04.

Addresses of Suppliers

Beckman Instruments, 12050 Tech Rd., Silver Spring, MD 20904

Dade Division, American Hospital Supply Corp., Miami, FL 33152

Fisher Scientific Co., 7722 Fenton St., Silver Spring, MD 20910

Gradko Glass Laboratories, Inc., 1 Bridge St., Yonkers, NY 10705

Hyland Division, Travenol Laboratories, Inc., Costa Mesa, CA 92626

Markson Science Supply, Del Mar, CA 92014

Matheson Coleman & Bell Chemicals, P.O. Box 85, E. Rutherford, NJ 07073

Scientific Products, 2175 V T., N.E., Washington, DC 20018

Sigma Chemical Co. P.O. Box 14508, St. Louis, MO 63178

Technicon Instruments Corp., Tarrytown, NY 10591

A.H. Thomas, Vone St. & Third, P.O. Box 779, Philadelphia, PA 19105-

VWR Scientific, Baltimore, MD 21224

By Order of the Secretary of the Army:

FRED C. WEYAND
General, United States Army,
Chief of *Staff*

Official:
VERNE L. BOWERS
Major General, United States Army
The Adjutant General

Distribution:
To be distributed in accordance with DA Form 12-34 (qty rqr block no. 13) requirements for TB MED Series: Professional Medical Material.

PIN: 009986-000